

WHAT IS CLAIMED IS:

1. A multiplexed enzyme assay method comprising:

performing a plurality of enzyme reactions in the presence of a set of enzyme
5 substrates, under conditions effective to convert an enzyme substrate in the set to a
corresponding product in the presence of the enzyme for that substrate, where (i) each
substrate and the product of that substrate have different separation characteristics from
each other and from the other substrates in the set and their corresponding products and
(ii) each substrate and its corresponding product have a detection moiety capable of
10 producing a detectable signal,

separating the substrates and products in said reactions in a single separation
medium,

detecting, for each separated product, a separation characteristic effective to
identify that product and a signal related to the amount of the product, and

15 determining, from the detected separation characteristic and signal detected for
each product, the amount of substrate converted to the corresponding product in each of
said reactions.

2. The method of claim 1, wherein the separation characteristic of said substrates and
20 products is electrophoretic mobility, and said separating includes separating the substrates
and corresponding products within an electrophoretic medium, under the influence of an
applied electric field.

3. The method of claim 2, wherein the substrates and corresponding products are
25 separated by capillary electrophoresis.

4. The method of claim 1, wherein said detecting further includes detecting, for
each of said substrates, a separation characteristic effective to identify that substrate and a
signal related to the amount of the substrate.

30 5. The method of claim 1, wherein said substrates and corresponding products are
fluorescently labeled, and said detecting includes detecting the fluorescent signal from each
product when irradiated with a fluorescence excitation wavelength.

6. The method of claim 1, wherein said plurality of enzyme reactions are carried out in a single reaction mixture containing a plurality of different enzymes, and each of said enzymes is effective to convert one of said substrates in said reaction mixture to the
5 corresponding product.

7. The method of claim 6, for use in determining the levels of activity of each of a plurality of different enzymes in a cell, under selected cell conditions, wherein said different enzymes in said reaction mixture are obtained from said cell under such selected cell
10 conditions.

8. The method of claim 7, for determining changes in the levels of activity of each of a group of enzymes in a cell, in control and test cell conditions, wherein said performing, separating, detecting, and determining steps are carried out for enzymes obtained from the
15 cells at the control and test conditions.

9. The method of claim 7, for determining changes in the levels of activity of each of a group of enzymes in a cell, when the cell is exposed to an agent known or being tested for its ability to inhibit or activate the level of the activity of one or more of said different
20 enzymes, wherein said performing, separating, detecting, and determining steps are carried out for enzymes obtained from the cells before and after exposure to said agent.

10. The method of claim 9, wherein said group of enzymes are selected from groups consisting of receptor-kinase enzymes and cell-signaling pathway enzymes.
25

11. The method of claim 1, for assaying the effect of one or more agents in inhibiting or stimulating the activity of a selected enzyme, wherein said performing step includes performing multiple separate enzyme reactions in separate reaction mixtures, where each mixture contains (i) one or more enzymes and (ii) one or more of the substrates
30 from said set, and wherein said performing step further includes combining said reaction mixtures prior to separating the substrates and products of the multiple reactions.

12. The method of claim 11, for use in screening for or evaluating a test compound capable of affecting enzyme activity, wherein the separate reaction mixtures also include one or both of (a) one of a plurality of different test agents being tested for its ability to activate or inhibit the activity of the enzyme, and (b) one of a plurality of different concentrations of a single agent.

13. The method of claim 11, wherein the different substrates in a set include (i) an enzyme substrate moiety, (ii) a mobility modifier that imparts a separation characteristic to each substrate and its corresponding product in the set that is unique with respect to the separation characteristics of other substrates and corresponding products in the set, and (iii) a detection moiety that permits detection of a signal from said substrates and their corresponding products.

14. The method of claim 13, wherein the different substrates in a set have an oligopeptide substrate moiety, and said mobility modifier is selected from the group consisting of (i) non-peptide moieties coupled to the oligopeptide, (ii) one or more amino acid substitutions in said oligopeptide that preserve recognition of said substrate moiety by said enzyme, but alter the separation characteristic of the oligopeptide, and (iii) different reporter moieties with different separation characteristics.

20

15. The method of claim 11, for assaying interactions between a receptor enzyme and a ligand known to affect the receptor-enzyme activity, wherein said separate enzyme reaction mixtures include (i) said enzyme (ii) one of the substrates from said set, (iii) one or more different concentrations of said ligand, including, in at least one reaction mixture, a concentration of ligand sufficient to produce optimal activation of the enzyme.

25

16. The method of claim 15, wherein at least one of the reaction mixtures contains substantially no ligand, to provide a enzyme activity level of non-activated enzyme.

30

17. The method of claim 15, for assaying the ability of one or more test agents to interfere with ligand-activated enzyme activity, where at least some of the reaction mixtures contain, in addition to an optimal concentration of ligand and one of a plurality of different test agents.

18. The method of claim 15, for assaying the effect of one or more test agents to interfere with ligand-activated enzyme activity, where at least some of the reaction mixtures contain an optimal concentration of ligand and one or more different concentrations of at least one test agent.

19. The method of claim 15, wherein said receptor enzyme is a receptor-kinase enzyme effective, in an activated state, to phosphorylate an oligopeptide substrate, and the substrates in said set include such oligopeptide substrate, a mobility modifier is selected from the group consisting of (i) non-peptide moieties coupled to the oligopeptide, (ii) one or more amino acid substitutions in said oligopeptide that preserve recognition of said substrate moiety by said enzyme, but alter the separation characteristic of the oligopeptide, and (iii) different detection moieties with different separation characteristics.

20. The method of claim 19, wherein said receptor enzyme is selected from the group consisting of EGFR, where the ligand is EGF, receptor II kinase, where the ligand is insulin, and ERK and insulin receptor kinase, where the ligand is insulin.

21. The method of claim 11, wherein said enzyme is a kinase.

22. The method of claim 21, wherein said enzyme is selected from the group consisting of ERK kinase, S6 kinase, P38 kinase, and Abl kinase.

23. A set of enzyme substrates for performing a multiplexed enzyme assay, comprising a plurality of enzymes substrates, each having (i) an enzyme substrate moiety at which an enzyme in the assay reacts with the substrate, to convert it to the corresponding product, (ii) a mobility modifier that imparts to each substrate and its corresponding product in the set a unique separation characteristic with respect to the separation characteristics of other substrates and corresponding products in the set, and (iii) a detection moiety that permits detection of a signal from said substrates and products in the set.

24. The set of claim 23, wherein the detection moiety is a fluorescent reporter.

25. The set of claim 23, for use in a method for assaying one or more enzymes having an oligopeptide substrate, wherein the substrates in a set have an oligopeptide substrate moiety, and said mobility modifier is selected from the group consisting of (i) non-peptide moieties coupled to the oligopeptide, (ii) one or more amino acid substitutions in said oligopeptide that preserve recognition of said substrate moiety by said enzyme, but alter the separation characteristic of the oligopeptide, and (iii) different detection moieties with different separation characteristics.
- 5